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Precorneal Clearance of Mucoadhesive Microspheres from the Rabbit Eye

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Abstract

The ocular disposition of hydrated ^{111}In-labelled microspheres was investigated in the rabbit by gamma scintigraphy. Microspheres of cross-linked poly(acrylic acid) (Carbopol 907) were prepared by a w/o emulsification process.

An in-vitro mucoadhesion test of prehydrated microspheres showed that greater adhesion was achieved to a mucus gel at pH 5·0 compared with pH 7·4.

Clearance was a biphasic process with a rapid initial phase preceding a slower basal phase. When hydrated in pH 5·0 phosphate buffered saline, clearance during the basal phase was slower compared with a pH 7·4 buffered preparation. Both prehydrated preparations were retained on the preocular area during the basal phase for longer periods than non-hydrated microspheres.

The retention on the ocular surface of approximately 25% of the instilled dose would suggest this technology will have application for controlled ophthalmic drug delivery.

A major research objective in ophthalmic drug delivery during the past two decades has been to design systems for prolonging the residence times of drugs in the conjunctival sac. Administration of ophthalmic drugs in aqueous solutions results in extensive drug loss, mainly due to tear fluid dynamics which occur in removing the solution from the eye (Lee & Robinson 1979). Only a small portion (1–3%) of the applied drug penetrates the cornea and reaches intraocular tissues (Patton & Robinson 1976). Thus, for certain drugs, frequent administration of eye drops is necessary to maintain an adequate drug concentration.

The addition of suitable polymers to liquid ophthalmic vehicles is a common method for increasing the ocular contact time and hence the drug bioavailability (Saettone et al 1982, 1984). Relatively low viscosity ophthalmic vehicles generally, however, do not have a sustaining effect. Ointments and inserts have been the main choice of formulations for achieving prolongation of drug action, although both have certain disadvantages including vision blurring and sticking of the lids. Gels or slowly-dissolving lamellae remain in the eye for extended periods of time, but their usual high water content allows drug substances to diffuse rapidly from the system (Patton & Robinson 1975; Bensinger et al 1976; Katz & Blackman 1977; Grass et al 1984). Certain new approaches have now been adopted to develop controlled drug delivery systems for the eye like liposomes (Singh & Mezei 1983; Stratford et al 1983; Guo et al 1988), nanoparticles (Wood et al 1985; Fitzgerald et al 1987) and microspheres (Beal et al 1984).

Recently, Thermes et al (1992) investigated the ocular bioavailability in albino rabbits of timolol in the presence of poly(acrylic acid) (PAA) by determination of drug concentrations in the cornea, aqueous humour, iris and ciliary body. The results were compared with 0·5% timolol in isoviscous solutions of poly(vinyl alcohol) (PVA) and timolol-poly(acrylic salt) (PAA salt). An increase in ocular bioavailability was achieved for all viscous formulations, although the largest increase was obtained with the non-mucoadhesive polymer PVA. The mucoadhesive polymer PAA changed the concentration vs time profiles of timolol and resulted in the highest timolol concentrations in the iris and ciliary body. These results are different from those reported by Davies et al (1991), who showed an improved bioavailability of pilocarpine when coadministered with PAA compared with an equiviscous solution of PVA.

Bioadhesive polymers may therefore be useful adjuvants for ocular formulations as they have been shown, when administered in particulate form, to improve the ocular bioavailability of progesterone (Hui & Robinson 1985) through non-covalent bond formation to the mucin layer of the conjunctival surface (Robinson 1989).

The objectives of this study were to produce some microparticulate mucoadhesive hydrogels (microspheres) composed of poly(acrylic acid) cross-linked with maltose. Such microspheres should rapidly hydrate and swell within the tear film and if bonding is possible with the ocular glycoproteins, increase the residence time in the precorneal area. Gamma scintigraphy was employed to investigate the influence of vehicle pH (5·0 and 7·4) on the clearance of prehydrated microspheres from the rabbit eye. Finally, we sought to study the influence of hydration state on the clearance process by comparing dry powder microspheres with the prehydrated systems.

Materials and Methods

The following materials were used as received: Carbopol 907 (450 kDa) (poly(acrylic acid), BF Goodrich Company, Akron, USA), olive oil, maltose, palmitic acid (Sigma Chemical Co., Poole, UK), ^{111}In as indium chloride (185 MBq) (NEN, Southampton). Mucus glycoprotein (BDH Chemicals Ltd, Poole, UK). All other reagents were of AnalaR grade (BDH Chemicals Ltd, UK).

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Preparation of microspheres

A water-in-oil emulsion was prepared comprising 50 mL aqueous phase (0·25 g Carbopol 907 and 0·125 g maltose dissolved in water) and 150 mL olive oil containing 0·5 g palmitic acid. The emulsion was agitated continuously with a mixer (Ultra-Turrax T25 Janke and Kunkel Ika-Labortechnik) at 8000 rev min^{-1} for 5 h at 115°C. The resultant microspheres were cured at 120°C for 1 h. The suspended microspheres were cooled and the oil phase separated before washing the microspheres several times with acetone to remove traces of oil. The microspheres were then dried at 60°C under vacuum.

Sizing of microspheres

A 2% suspension of microspheres in distilled water was viewed under a light microscope (Olympus BH-2); 90% of microspheres were in the range 8–15 μm.

Labelling of microspheres

Indium-111 solution (0·2 mL prepared by mixing 1 mL of $^{111}InCl_3$ (70 MBq) with 30 μL of acidic sodium acetate solution (0·05% w/v)) was added to 20 mg microspheres which were mixed for 30 min before drying at 60°C under vacuum for 1 h. The labelling efficiency was determined by suspending the microspheres in phosphate buffer solution at pH 5·0 and 7·4 for 3 h. The suspension was then centrifuged and the supernatant analysed by gamma counting (LKB 1282 Compugamma CS) for the activity dissociated.

In-vitro mucoadhesion determination

The mucoadhesive properties of the microspheres were evaluated using the method of Hunt (1988) (Fig. 1). The

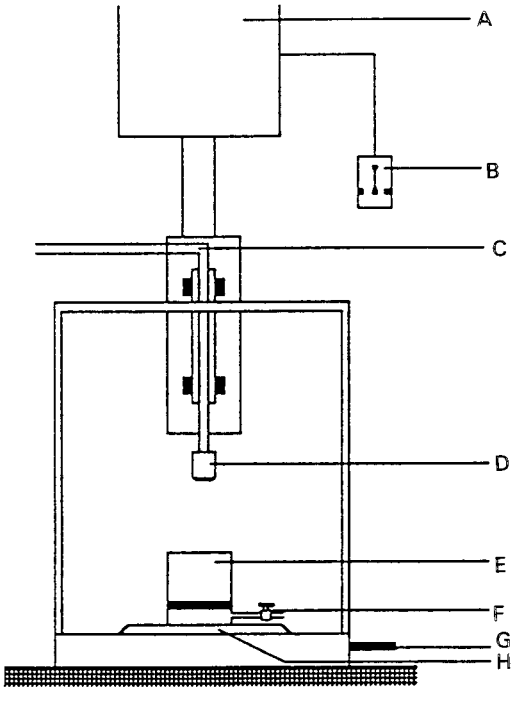

FIG. 1. Schematic diagram of mucoadhesion testing apparatus. A syringe pump, B remote control for syringe pump operation, C vacuum line, D upper probe, E lower cell, F vacuum line, G data output to micro computer, H balance pan.

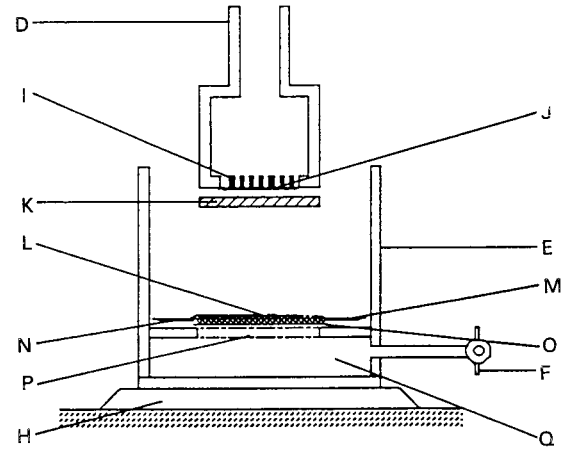

FIG. 2. Schematic diagram of mucoadhesion cell. D upper probe, E lower cell, F vacuum line, H balance pan, I perforated end piece, J filter paper, K microspheres (hydrated), L mucus, M polythene ring, N white petroleum jelly, O filter paper, P perforated end piece, Q evacuated chamber.

mucin gel (0·3 g containing 20% mucus glycoprotein) was spread evenly across an 18-mm diameter cellulose nitrate membrane (SM 11107, 0·01 μm, Sartorius) which was held in place on the upper probe by a vacuum (Fig. 2). A cellulose acetate membrane (0·2 μm) was placed over the perforated section of the lower section of the cell and 0·2 mL microsphere suspension spread to form a uniform film over the surface of the membrane. This section of the cell was evacuated and clamped to the balance pan. The upper probe was lowered to touch the microsphere film on the lower filter for 20 s. The upper probe was then raised and the transmitted force measured to determine the force of detachment.

Precorneal clearance study

Each formulation was tested in a cross-over manner in a group of four male New Zealand White rabbits (3–4 kg) with a minimum wash-out period of three days. The rabbit was positioned 5 cm away from the 3·5-mm aperture of the pinhole collimator of the gamma camera (IGE Maxi Gamma 400A). The radiolabelled (1·5 MBq) suspension (20 μL) or drug microsphere preparation was deposited directly onto the corneal surface and the eye manually blinked once to distribute the formulation over the cornea. Time frames of 20 s were used for the first 5 min increasing in length to 2 min frames for the last 14 min of the dynamic study. This was followed by 60-s static images taken every 30 min. Images were recorded up to 167 min post-instillation. Regions of interest were created around the images of the cornea, the inner canthus and the lacrimal duct. Graphs of activity remaining in each region vs time were plotted after correction for background to assess the clearance from the corneal surface.

Results and Discussion

In-vitro mucoadhesion study

Table 1 shows the force required for the detachment of the prehydrated microspheres at pH 5·0 and 7·4 from the model

Table 1. In-vitro evaluation of Carbopol 907 bioadhesion to mucus glycoprotein.

Test material	Weight required for detachment (g)	Force (N)	Force/area ($N\,cm^{-2}$)
pH 5·0			
Microspheres	30·55 ± 1·54	0·30 ± 0·015	1·19 ± 0·06
Carbopol 907 gel	45·72 ± 5·79*	0·45 ± 0·056*	1·79 ± 0·22*
pH 7·4			
Microspheres	17·71 ± 3·44*	0·17 ± 0·031*	0·68 ± 0·12*
Carbopol 907 gel	26·97 ± 8·50†	0·26 ± 0·083†	1·04 ± 0·33†

*$P < 0.05$ compared with microsphere pH 5·0 experiments. †$P < 0.05$ compared with Carbopol pH 5·0 experiments.

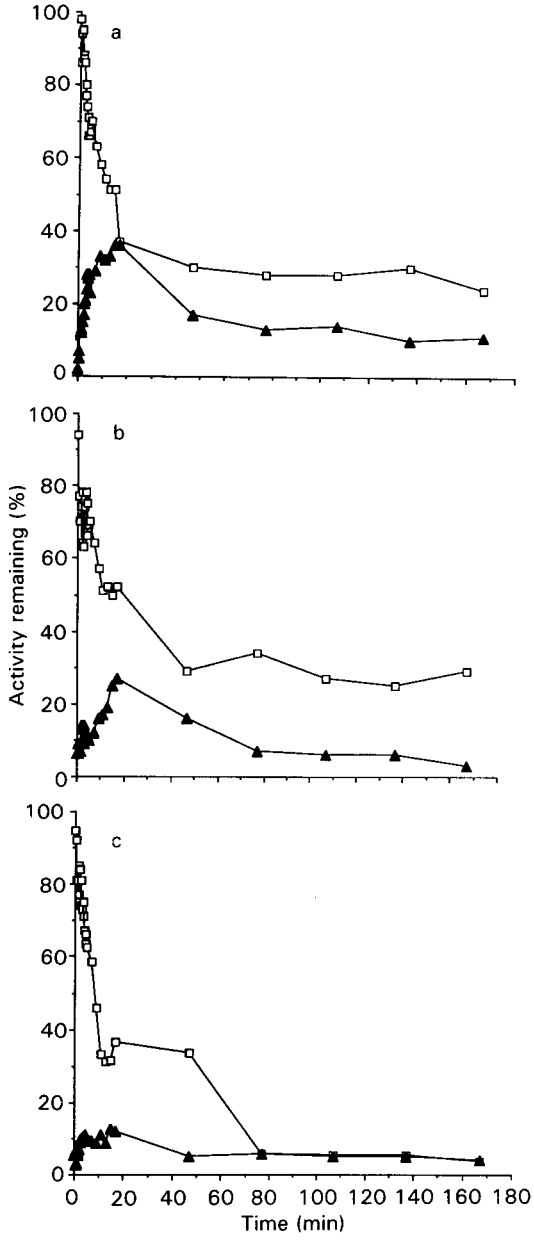

FIG. 3. Precorneal clearance of microspheres in suspension at pH 5·0 (a), pH 7·4 (b) and in powder form (c). Regions of interest: □ cornea; ▲ inner canthus.

mucus gel. For comparison purposes, results are also tabulated for a 0·25% w/v gel of Carbopol 907. The Carbopol 907 gels were more mucoadhesive than the microsphere samples. For both gels and microspheres, the force of detachment was greater for the pH 5·0 formulations compared with those at pH 7·4 ($n = 4$, $P < 0.05$). At pH 5·0 the presence of protonated carboxyl groups permits enhanced bioadhesion due to hydrogen-bond formation with the hydroxyl groups of the glycoprotein molecules.

Precorneal clearance study

The labelling efficiency was 95–98%. The precorneal clearance of microspheres in suspension form at pH 5·0 and 7·4 is shown in Fig. 3a and b, respectively. pH had no statistical effect on AUC or $t_{\frac{1}{2}}$ (analysis of variance). The clearance of microspheres administered as a dry powder (Fig. 3c) was more rapid than in the hydrated form, probably due to incomplete hydration of the former in the tear film. Table 2 summarizes microsphere clearance parameters for suspension forms at pH 5·0, pH 7·4 and in the dry state.

The clearance of microspheres from the ocular surface followed a biphasic process with an initial rapid phase followed by a much slower basal phase. Similar profiles have been reported for other colloidal carriers e.g. liposomes (Davies et al 1992). Table 3 summarizes the kinetic parameters obtained by fitting the data to a biexponential equation. The initial clearance phase (k_1) was independent of pH and hydration state; however, a significant difference was found between the k_2 values of the pH 5·0 and 7·4 formulations ($P = 0.043$). The dry microspheres ($k_2 = 0.06\,min^{-1}$) were found to clear more rapidly than the pH 5·0 suspension ($k_2 = 0.007\,min^{-1}$). This result is somewhat unexpected, given recent evidence of the contribution of mucus gel dehydrated by dry poly(acrylic acid) to the mucoadhesive process (Mortazavi & Smart 1993). The control of pH is therefore more critical to the ocular clearance process than the hydration state of the polymer.

Gamma scintigraphy had been previously employed by Beal et al (1984) to assess the clearance of polyphthalamide microcapsules containing ^{99m}Tc-labelled albumin from the rabbit eye. Sixty per cent of the instilled mass was found on the ocular surface after 90 min, which compared with only 10% of the aqueous albumin remaining after 10 min. In contrast, the present study resulted in 50% microsphere clearance after 14 min and at the termination of the study after 167 min, 24 ± 5·5% (pH 5·0) and 29 ± 12·7% (pH 7·4) of the activity remained (Table 2). The retention on the ocular surface after 167 min of approximately 25% of the instilled dose would suggest that this technology may have application for controlled ophthalmic drug delivery.

It is interesting to compare the ocular clearance of the microspheres with other mucoadhesive and colloidal delivery systems. Davies et al (1991) investigated the clearance of Carbopol 934P gel using ^{113m}In-DTPA complex and found a clearance rate of $5{\cdot}10\,min^{-1}$ for the initial and $0{\cdot}01\,min^{-1}$ for the basal phase. In the present study the clearance of microspheres in the initial phase was much slower ($k_1 = 0{\cdot}28$–$0{\cdot}31\,min^{-1}$). However, clearance in the basal phase was more rapid with rates of $0{\cdot}034\,min^{-1}$ and $0{\cdot}06\,min^{-1}$ for pH 7·4 and dry microspheres, respectively. Moreover, the microsphere suspension at pH 5·0 cleared

Table 2. Summary of in-vitro mucoadhesion and clearance parameters (± s.e.).

Test material	Force of detachment ($N cm^{-2}$)	% activity remaining (5 min)	% activity remaining (167 min)	Relative AUC	$t_{\frac{1}{2}}$ (min)
Microspheres at pH 5·0	1·19 ± 0·06	70·2 ± 15·6	24 ± 5·5	1·00	14 ± 6·0
Microspheres at pH 7·4	0·68 ± 0·12†	70·8 ± 8·4	29 ± 12·7	0·97	12 ± 6·4
Dry microspheres	—	62·6 ± 6·5	4·5 ± 0·7*	0·29	5·7 ± 2·2

*$P < 0.05$ compared with microspheres at pH 5·0 or pH 7·4. †$P < 0.05$ compared with microspheres at pH 5·0.

Table 3. Kinetics of clearance parameters of three different formulations of microspheres from the corneal region of the rabbit (n = 4, mean ± s.e.m.).

	Microspheres at pH 5·0	Microspheres at pH 7·4	Dry microspheres
A	71·75 ± 2·55	74·20 ± 2·32	74·58 ± 3·07
k_1 (min^{-1})	0·28 ± 0·033	0·30 ± 0·036	0·31 ± 0·052†
B	30·43 ± 2·64	26·49 ± 2·12	23·94 ± 3·93
k_2 (min^{-1})	0·007 ± 0·001	0·034 ± 0·01†	0·06 ± 0·010†

†$P < 0.05$ compared with microsphere suspension at pH 5·0. Data were fitted to the equation: $y = Ae^{-k_1 t} + Be^{-k_2 t}$.

more slowly in the basal phase compared with pH 7·4 and dry microspheres. Similarly, the clearance rate of the microsphere suspension and powder in the initial phase was much slower than the rate of clearance of hyaluronic acid ($P = 0.032$) (Durrani et al 1995). Comparing the initial (k_1) kinetic parameters of drainage from the corneal region at pH 5·0 with that of the liposomal clearance rates reported by Davies et al (1992), it was found that Carbopol 1342-coated liposomes ($k_1 = 1.23\ min^{-1}$) and Carbopol 934P-coated liposomes ($k_1 = 1.42\ min^{-1}$) cleared more rapidly than microspheres used in this study. Similar trends were shown at pH 7·4. The second, or basal phase (k_2) of the corneal drainage is a much slower phase but is a little faster at pH 7·4 compared with pH 5·0. The basal phase of corneal drainage depends on several processes, largely tear turn-over, conjunctival absorption and reflux between compartments.

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